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INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Peck, Tim L.	Mahomet	IL	US	
Olson, Dean	Champaign	IL	US	
Norcross, Jim	Champaign	IL	US	
Strand, David	Sherborn	MA	US	
Sweedler, Jonathan	Urbana	IL	US	

US-CL-CURRENT: 324/321; 324/306, 435/4

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INCORPORAT\$4	0
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Pre-Grant Publication (PGPub) Document Number (1):
20020149369

Summary of Invention Paragraph (5):

[0004] Systems for biochemical, chemical, and molecular analysis can be miniaturized as capillary-based systems or substrate-based, i.e., micro-scale, systems with multifunctional capabilities including, for example, chemical, optical, fluidic, electronic, acoustic, and/or mechanical functionality. Miniaturization of these systems offers several advantages, including increased complexity, functionality, and efficiency. Devices can be fabricated from diverse materials including, for example, plastics, polymers, metals, silicon, ceramics, paper, and composites of these and other materials. Mesoscale sample preparation devices for providing microscale test samples are described in U.S. Pat. No. 5,928,880 to Wilding et al. Devices for analyzing a fluid sample, comprising a solid substrate microfabricated to define at least one sample inlet port and a mesoscale flow channel extending from the inlet port within the substrate for transport of a fluid sample are described in U.S. Pat. No. 5,304,487. Currently known miniaturized fluid-handling and detection devices have not met all of the needs of industry.

Summary of Invention Paragraph (7):

[0006] NMR microcoils are known to those skilled in the art and are shown, for example, in U.S. Pat. No. 5,654,636 to Sweedler et al., and in U.S. Pat. No. 5,684,401 to Peck et al., and in U.S. Pat. No. 6,097,188 to Sweedler et al., all three of which patents are incorporated herein by reference in their entireties for all purposes. A solenoid microcoil detection cell formed from a fused silica capillary wrapped with copper wire has been used for static measurements of sucrose, arginine and other simple compounds. Wu et al. (1994a), J. Am. Chem. Soc. 116:7929-7930; Olson et al. (1995), Science 270:1967-1970, Peck (1995) J. Magn. Reson. 108(B) 114-124. Coil diameter has been further reduced by the use of conventional micro-electronic techniques in which planar gold or aluminum R.F. coils having a diameter ranging from 10-200 μm were etched in silicon dioxide using standard photolithography. Peck 1994 IEEE Trans Biomed Eng 41(7) 706-709, Stocker 1997 IEEE Trans Biomed Eng 44(11) 1122-1127, Magin 1997 IEEE Spectrum 34 51-61, which are also incorporated herein by reference in its entirety for all purposes.

Summary of Invention Paragraph (8):

[0007] Miniature total analysis systems (μ -TAS) are discussed in Integrating Microfluidic Systems And NMR Spectroscopy--Preliminary Results, Trumbull et al, Solid-State Sensor and Actuator Workshop, pp. 101-05 (1998), Magin 1997 IEEE Spectrum 34 51-61, and Trumbull 2000 47(1) 1-6 incorporated herein by reference in its entirety for all purposes. The Trumbull et al. device integrated multiple chemical processing steps and the means of analyzing their results on the same miniaturized system. Specifically, Trumbull et al. coupled chip-based capillary electrophoresis (CE) with nuclear magnetic resonance spectroscopy (NMR) in a μ -TAS system.

Summary of Invention Paragraph (9):

[0008] Capillary-based liquid chromatography and microcoil NMR have compatible flow rates and sample volume requirements. Thus, for example, the combination of the Waters CapLC.TM. available from Waters Corporation (Milford, Mass., USA) and the MRM CapNMR.TM. flow probe available from MRM Corporation (Savoy, Ill.), a division of

Protasis Corporation (Marlborough, Mass., USA) provides excellent separation capability in addition to UV-VIS and NMR detection for mass-limited samples. The Waters CapLC.TM. has published flow rates from 0.02 $\mu\text{L}/\text{minute}$ to 40 $\mu\text{L}/\text{minute}$. A typical CapLC on-column flow rate is 5 $\mu\text{L}/\text{min}$, the autosampler-injected analyte volume is 0.1 μL or more, and accurate flow rates are achieved through capillary of typically 50 μm inner diameter. The NMR flow cell has a typical total volume of 5 μL with a microcoil observe volume of 1 μL . A typical injected sample amount for CapLC-NMR analysis is a few μg (nmol) or less.

Summary of Invention Paragraph (10):

[0009] Capillary scale systems also are shown in U.S. Pat. No. 6,194,900, the entire disclosure of which is incorporated herein by reference for all purposes. In such systems, a capillary-based analyte extraction chamber is connected to an NMR flow site, such as by being positioned as an operation site along a capillary channel extending to the NMR flow cell.

Summary of Invention Paragraph (11):

[0010] Small volume flow probes are shown, for example, by Haner et al. in Small Volume Flow Probe for Automated Direct-Injection NMR Analysis: Design and Performance, J. Magn. Reson., 143, 69-78 (2000), the entire disclosures of which is incorporated herein by reference for all purposes. Specifically, Haner et al show a tubeless NMR probe employing an enlarged sample chamber or flowcell. Microcoil-based micro-NMR spectroscopy is disclosed in U.S. Pat. No. 5,654,636, U.S. Pat. No. 5,684,401, and U.S. Pat. No. 6,097,188, the entire disclosures of all of which are incorporated herein by reference for all purposes. Sample amounts can now range as small as several hundred microliters for conventional flowprobes to smaller than 1 μL for microcoil-based capillary-scale flowprobes. Acquisition times typically range from minutes to hours. The most expensive and technologically limiting component of the NMR system is the superconducting magnet. Although significant financial and technical investment has been made in the development of elaborate mechanical (robotic-controlled) sample changers and, more recently, automated flow injection systems for repetitive and continuous sample throughput, the magnet remains today a dedicated component in which only sequential, one-at-a-time analysis of samples is carried out.

Detail Description Paragraph (2):

[0034] It will be recognized by those skilled in the art that numerous different embodiments of the systems, probes and modules disclosed here can be produced and used for various different applications. In capillary-based embodiments analyte sample fluid has a fluid flow rate typically less than about 5 $\mu\text{L}/\text{minute}$. In substrate-based, i.e., micro-scale, embodiments the analyte sample fluid has a fluid flow rate typically less than 1 $\mu\text{L}/\text{minute}$. In certain preferred embodiments, a miniaturized analysis system is employed for liquid phase sample analysis. Such embodiments, referred to in some instances here and in the appended claims as substrate-based, employ a microfabricated support body or manifold in the form of a cylinder, chip, laminated planar substrate or the like, insertable, e.g., removeably insertable, such as a set of interchangeable modules or the like. Typically in such embodiments the module has one or more straight or branched microfabricated microchannels and the probe has an inlet port for feeding fluid from an external source into the manifold. Multiple NMR detection sites each comprising an NMR RF microcoil in the module are in fluid communication with the inlet via one or more of the microfabricated microchannels. As used here, the terms "micro-scale" and "microfluidic" means the manifold operates effectively on micro-scale fluid samples, typically having volumes less than about 1 μL (i.e., 1 microliter), e.g., about 0.1 microliter to 1.0 microliter, and fluid flow rates less than about 1 $\mu\text{L}/\text{min}$, for example 100 nanoliters/min. The term "microscale" also refers to flow passages or channels and other structural elements of a substrate, e.g., a multi-layer laminated substrate. For example, 1 or more microchannels of a module substrate preferably have a cross-sectional dimension (diameter, width or height) between 500 microns and 100 nanometers. Thus, at the small end of that range, the microchannel has cross-sectional area of about 0.01 square microns. Such microchannels within a laminated substrate of the module, and chambers and other structures within the laminated substrate, when viewed in cross-section, may be triangular, ellipsoidal, square, rectangular, circular or any other shape, with at least one and preferably all of the cross-sectional dimensions transverse to the path of fluid flow is microscale. It should be recognized, that one or more layers of a laminated substrate may in certain embodiments have operative features, such as fluid channels, reaction chambers or zones, accumulation sites etc. that are larger than microscale. The modules disclosed here provide effective microcoil NMR devices and

systems with good speed of analysis, decreased sample and solvent consumption, increased detection efficiency, and in certain embodiments disposable fluid-handling devices.

Detail Description Paragraph (4):

[0036] Referring now to the drawings, FIG. 1 is a schematic representative of an electrofluidic system in accordance with the present disclosure. A multiplicity of sample management modules are in operative electrical and fluidic communication with a multiplicity of primary stage detectors, a peak management module, and a multiplicity of ancillary stage detectors. Sample introduction can be from a variety of introduction means well known to those skilled in the art, and may include autosamplers with or without additional means of solid phase extraction. In general, the flow of information and fluid transport depicted in FIG. 1 can proceed in either direction. For example, with the appropriate plumbing as understood by those skilled in the art, a storage loop used for sample introduction can be reused for sample storage, e.g. as a fraction collector at the end of the experiment. Furthermore, the figure should be considered sufficiently general as to represent the combination of any number of individual components, for example, the case where a single sample management module is used with a multiplicity of ancillary stage detectors. The sample management platforms are sufficiently sophisticated to be in operative electrical and fluidic communication with each other. One embodiment of this configuration is where each of the detection stages are NMR microcoil detectors. A preferred embodiment is where all detectors are integrated into the probe manifold but are not limited to NMR, e.g. UV, IR, and other NMR-compatible (predominantly non-magnetic) means of detection. The peak management module can include sample storage and routing capabilities, but can also include a means of sample management, e.g. solid phase extraction. In a most preferred embodiment, the components shown are predominantly integrated into a probe module, such as those of FIGS. 3-8, with intelligent control of the overall processes being directed at least in part by electrical and fluidic processing elements in (i.e., on-board) the probe, e.g. microprocessors in operative communication with the detectors and fluidic management components shown in the drawings.

Detail Description Paragraph (8):

[0040] Referring to FIG. 12, a laminated substrate of a substrate-based module is seen to comprise, a first plastic piece 10 and a second plastic piece 11 welded together by selective IR irradiation of either the plastic pieces or by irradiation of an optional EM absorbing substance 12. The substrate contains a channel 13 formed by welding of the two plastic pieces together. Optionally contained within the channel 13 is an environmentally sensitive element 14. The substrate may also contain other channels formed from welding the plastic pieces together. A second channel 15 is in close and continuous contact with an embedded microdevice 16. A port 17 provides communication from the channel to the top or bottom planar surface of the substrate. Additionally, an external device may be connected to the fluid-handling substrate through the port. An optional gasket 18 may be used to enhance the fluid-tight seal around the channel. An optional EM absorbing layer 19 may be placed anywhere along the surface of the substrate. FIG. 13 shows cross-sectional views of four alternative configurations for fluid channels in the probe module. Such channels may be formed, for example, by welding module layers e.g. plastic pieces together. Possible configurations include, but are not limited to, semi-circular 21, rectangular 22, rhomboid 23, and serpentine 24. The channel configurations are limited only by the thickness of the materials forming the fluid-handling substrate.

Detail Description Paragraph (10):

[0042] The module may be any size and shape suitable to the intended application. In a preferred embodiment the module may be placed in a housing that forms a housing of the probe. In certain embodiments the probe is tubular or finger shaped and/or boxed-shaped to match the representative forms of NMR probes, with for example, a diameter of about one inch and a height of about 20-30 inches. In certain embodiments the module is generally planar. The term "generally planar" means card or cartridge-like, optionally being curvo-planar or otherwise irregular, but otherwise typically being rectangular or right-cylindrical, and having a thickness less than about a third, preferably less than one quarter, more preferably less than one fifth e.g. about one sixth or less, the largest dimension of major (i.e. largest) surface of the substrate. Other embodiments will be apparent given the benefit of this disclosure.

Detail Description Paragraph (11):

[0043] The microfluidic nature of the NMR probe modules disclosed here provides significant commercial advantage over conventional (larger scale, e.g. larger than capillary) fluidic NMR systems. Less sample fluid is required, which in certain applications can present significant cost reductions, both in reducing product usage (for example, if the test sample is taken from a product stream) and in reducing the waste stream disposal volume. In addition, the microfluidic substrate assemblies can, in accordance with preferred embodiments, be produced employing MEMS and other known techniques suitable for cost effective manufacture of miniature high precision devices.

Detail Description Paragraph (12):

[0044] The module may be made of any number of materials. Examples include metals, plastics, and silica. In a preferred embodiment the manifold comprises a substrate formed at least in part of polyetheretherketone (PEEK). PEEK is a high temperature resistant thermoplastic. PEEK has superior chemical resistance allowing for its use in harsh chemical environments, and it retains its flexural and tensile properties at very high temperatures. Additionally, glass and carbon fibers may be added to PEEK to enhance its mechanical and thermal properties. In another embodiment the module is a multi-layered laminate. Other embodiments will be apparent to one skilled in the art given the benefit of this disclosure.

Detail Description Paragraph (14):

[0046] The fluid inlet port is where a fluid sample to be tested is introduced to the module. The fluid inlet port is typically sized to accommodate the amount of sample being tested. The inlet port may have any number of shapes. Examples include circular, square, trapezoidal, and polygonal. The inlet port may or may not also include additional filtering for the fluid sample to be tested. In certain embodiments there may be multiple inlet ports. Further embodiments will be apparent to one skilled in the art given this disclosure.

Detail Description Paragraph (15):

[0047] The fluid pathway transports the fluid sample to be tested through-out the module. In a preferred embodiment where the probe module is microfluidic the scale the multiple channels (sometimes referred to as microchannels) may be capillary in scale. The orientation of the channels may be any number of configurations. Examples include parallel, intersecting, overlapping, spiral, serpentine, and circular. The cross section of the channels may have any number of shapes as well. Examples include circular, square, trapezoidal, and polygonal. Further embodiments of size, shape, and orientation will be apparent to one skilled in the art given the benefit of this disclosure.

Detail Description Paragraph (16):

[0048] The multiple NMR sites are provided to allow for increased functionality and/or throughput. With multiple NMR sites the user is able to perform multiple NMR tests simultaneously which increased the rate in which results may be obtained. Furthermore the NMR detection sites may be optimized for different types of testing allowing a single probe to be used for a number of tests. In some embodiments each NMR site maybe in fluid communication with multiple channels of the fluid pathway. In accordance with preferred embodiments, the NMR detection sites further comprise matching capacitors and tuning capacitors, fluid connectors and data transmission means such as signal carrying leads or the like. An example of an NMR detection site can be seen in FIG. 14. Other embodiments will be apparent to one skilled in the art given the benefit of this disclosure.

Detail Description Paragraph (18):

[0050] In a preferred embodiment the sample holding void is cylindrical in shape but it may also have any number other shapes, most notably spherical. Examples of cross-sectional configurations include round, rectangular, triangular, etc. In a preferred embodiment the sample holding void is 5 um to 500 um, more preferably 25 um to 50 um. In a preferred embodiment the microcoil surrounds around a portion of the void. Preferably the microcoil is made of copper but may be made of any number of other conductive or super-conductive materials depending on the desired properties. The microcoil typically is 250 um to 1 mm in axial direction. In preferred embodiments the microcoil may be helical, solenoidal or spiral and in other preferred embodiments the microcoil may be planar. Other embodiments of coil geometry will be apparent to one skilled in the art given the benefit of this disclosure.

Detail Description Paragraph (19):

[0051] In the NMR probe module configuration disclosed here, each of the NMR detection sites is separate and therefore, optionally, can hold unique samples for testing. In this regard, the probe modules integrate a multiplicity of detectors with greater functionality enhancement than would be achieved by wrapping a multiplicity of detection coils around a single sample. The microcoil, void, and sample are magnetically matched, and the NMR detection sites in accordance with preferred embodiments are operative to obtain high resolution NMR spectra. The microcoil is positioned to within 1 mm, more preferably to within less than 100 μ m of the sample boundary. The incorporation of multiple microcoils and multiple corresponding voids complicates magnetic matching, but will be within the ability of those skilled in the art given the benefit of this disclosure and applying known principles. Preferably, at least one detection coil is optimized for high resolution proton detection, whereas other coils may be optimized for heteronuclear or multi-dimensional homonuclear experiments. In two-dimensional experiments such as correlation spectroscopy (COSY) or total correlation spectroscopy (TOCSY), and in heteronuclear experiments the digital resolution in the f1 dimension is relatively coarse (typically 128-256 data points). The number of data points in the f2 dimension (typically 512) is, therefore, considerably reduced compared to one-dimensional experiments, and the acquisition time is similarly reduced (.about.200 ms at 250 MHz, and shorter at higher operating frequencies). Consequently, the resolution requirements of coils intended for 2-D acquisition is considerably lower due to the larger spectral linewidths (typically 2-4 Hz) in 2-D experiments. For example, in an embodiment with 2 detection sites, a primary coil can be optimized for resolution while a secondary coil can be optimized for heteronuclear acquisition.

Detail Description Paragraph (24):

[0056] Li et al. (Li 1999 Anal. Chem. 71 4815-4820) describes a 4-coil assembly illustrative of certain aspects of the present disclosure. The solenoidal microcoils (diameter=360: μ m, length approx. 1 mm) are mounted on horizontal (transverse to B₀) capillaries with a 90 degree rotation (x, y) and 5 mm vertical spacing between adjacent coils. Additional details of basic construction are known generally, as shown in the Li et al reference mentioned above and incorporated herein by reference for all purposes. The NMR probe modules disclosed here differ from such earlier devices in having multiple detectors, each having a detection site, i.e., as described above, void in the capillary microchannel to receive a test sample, and having an NMR microchannel aligned therewith.

Detail Description Paragraph (25):

[0057] It is generally preferred to immerse the diamagnetic wire of the microcoil in a diamagnetic matching medium with magnetic susceptibility equal to that of the wire. Suitable software is commercially available for use in determining determine inter-coil spacing, coil orientations, and other geometrical tradeoffs for optimum resolution, for example, Maxwell 3D (Ansoft Corporation, Pittsburgh, Pa.). Preferably, greater than 30 dB RF isolation is achieved between coils. Coil-to-coil coupling would result in crosstalk and interference in the received signals. RF coupling is typically minimized by geometrically positioning electrical components so that the orientation of the magnetic and/or electric fields in adjacent components are orthogonal. Adjacent components are also placed as far apart as is practical to maintain functionality but minimize coupling.

Detail Description Paragraph (28):

[0060] The microchannels and associated NMR microcoils can be formed in a module, preferably a multi-layer substrate, such as a laminated multi-layer substrate, e.g., a selectively welded multi-layer substrate as disclosed in copending U.S. patent application Ser. No. 60/239,010 filed on Oct. 6, 2000, the entire disclosure of which is incorporated herein by reference for all purposes. Microlithographic microcoils can be employed in such laminate substrates, such as those disclosed in the above-mentioned U.S. Pat. No. 5,684,401, the entire disclosure of which is incorporated herein by reference for all purposes. Alternatively, or in addition, one or more of the multiple NMR detector sites formed in the probe can be formed in a finger or peninsula-type extension of the substrate, and the microcoil can be formed as a separate 3-dimensional structure fitted over such substrate projection. It will be within the ability of those skilled in the art, that is, those skilled in this area of technology, given the benefit of this disclosure, to employ alternative suitable fabrication techniques for production of the multi-microcoil NMR detection probes disclosed here.

Detail Description Paragraph (29):

[0061] The controllable fluid router, which may be referred to as a form of a sample management engine, is operative in response to an electrical input signal to direct fluid sample in the module to at least a selected one of the multiple channels corresponding to the input signal. The fluid router may direct all or part of the fluid sample to one, multiple, or all of the channels in the fluid pathway. There are many ways the router could be implemented. For example, in one embodiment the router may consist of individual valves for each channel of the fluid pathway that are individually controlled by an electrical input signal. In another embodiment the router may perform cross routing of sample from one channel to another and be controlled entirely by one input signal. In a preferred embodiment the fluid router is a sample management engine to selectively deliver and, optionally, selectively withdraw fluid samples from the microchannels of the probe. Preferably samples are fed into the microchannel of one or more of the probe's multiple detectors at a flow rate sufficiently low to maintain laminar flow. In another embodiment, the fluid routing functionality can be incorporated into one or more sample management modules. Other embodiments will be apparent to one skilled in the art given the benefit of this disclosure.

Detail Description Paragraph (30):

[0062] In accordance with another embodiment the controllable router receives the electrical input signal from a controller unit. The controller unit may be any number of devices including, for example, a circuit, computer, microprocessor or microcontroller. The controller unit may be located remotely or be incorporated in the module. The controller may optionally be connected to various sensor units as discussed in this specification. The input signal delivered by the controller unit may be software or hardware generated. The controller may or may not be in direct or indirect communication with the NMR sites.

Detail Description Paragraph (31):

[0063] In accordance with another embodiment the NMR sites are in communication with a data processing unit. The data processing unit may be any number of devices including, for example, a circuit, computer, or microprocessor. The data processing unit may be located remotely or be incorporated in the module. The data processing unit may optionally be connected to various sensor units as discussed in this specification. The controller may or may not be in direct or indirect communication with the fluid router.

Detail Description Paragraph (32):

[0064] In some embodiments the module probe may have both the controller unit and the data processing unit. In other embodiments the controller unit and data processing unit are in communication with each other. In still other embodiments one device, for example, a computer performs the functions of both. In another example, a microprocessor incorporated into the module performs the functions of both devices. Other embodiments will be apparent to one skilled in the art given the benefit of this disclosure.

Detail Description Paragraph (34):

[0066] A wide array of sample management forms may be employed in operative communication with the probe. The probe module may operatively communicate with a larger system that performs sample preparation. In one embodiment the module is connected to or part of a liquid chromatography system. In another embodiment the module is connected to or part of a capillary electrophoresis system. In another embodiment the module is connected to or part of a dynamic filed gradient focusing system. In some embodiments the sample preparation may also be incorporated in the probe module as discussed in this specification. In certain preferred embodiments wherein the channels and associated NMR microcoils are formed in a module, a liquid feed line (inside diameter preferably 5 microns to 500 microns, more preferably 25 to 50,) may connect to a port sited in the surface of the module from any of numerous commercially available sample management engines can be used, such as the Capillary Liquid Chromatography system from Waters Corporation, Milford, Mass., USA. As discussed further below, capillary-LC/micro-NMR systems are especially preferred embodiments of the system aspect of this disclosure. Peaks and/or samples, preferably already conditioned, purified, concentrated, etc., coming into the probe from the separation engine would be routed accordingly. Examples of such systems can be seen in FIG. 2.

Detail Description Paragraph (39):

[0071] The operative component can be any number of devices that interact with the fluid in the module including, for example, sensors, sample preparation devices,

pumps, heaters, coolers, ultrasonic devices and even additional NMR sites. The operative component may also be any number of devices that do not interact with the fluid. Examples, for instance, include microprocessors, micro-controllers and memory module. In some embodiments the operative component is in electrical communication with the controllable gate. In certain preferred embodiments the operative component is incorporated into the probe, e.g., integrated on-board the module. In other preferred embodiments the operative component is selectively removable. Having the operative component selectively removable allows for swapping of different operative components allowing for greater configuration flexibility. In other embodiments the operative component is in communication with the one or more of the NMR detector sites. The operative device may also optionally in communication with a controller unit, a data processing unit or both. In other embodiments there may be multiple operative components in any of the configuration discussed above or below, which may or may not be in communication with each other. Other embodiments will be apparent to one skilled in the art given the benefit of this disclosure.